Oral Aspects of Steviosides

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Univ.-Prof. Dr. Matthias Frentzen
Oral Aspects of Steviosides - Content

- The teeth and their environment
- The oral cavity as an ecological network
- Oral biofilms / dental plaque
- Oral diseases
- Strategies to prevent biofilm pathology
- Sugar substitutes in dentistry
- History and literature review on Steviosides in dental research
- Preliminary results of in-vitro tests
- Estimated advantages of Steviosides in dentistry
The oral cavity is the space in the mouth behind the gums and teeth. It is bounded above by the hard and soft palates and below by the tongue and mucous membrane, connected to the inner part of the mandible.

Major health problems:
- Tooth Decay
- Periodontal Disease
- Cancer
- Cranio-mandibular Disorders (Orthodontics)
Microbial biofilms form on oral surfaces, which usually exist in dynamic equilibria with host defenses, and are compatible with maintenance of the integrity of target tissues. The populations consisting of communities of oral microbial residents are not equally distributed throughout the oral cavity, but rather on different sites of various, respectively, predominant microflora. The lips build the border between the skin microflora which consists predominantly of staphylococci, micrococci and gram-positive rods (e.g. Corynebacterium, Propionibacterium spp.), and the mouth which contains streptococci and many anaerobic gram-negative types.
Saliva: Although saliva contains up to 10^9 microorganisms/mL, it is not expected to have a residential microflora. The normal swallowing process prevents the bacteria from reproducing and thus remaining in the oral cavity.

The microbial community associated with teeth (biofilm) is composed differently due to the various local environmental conditions on each tooth surface.
Central to the initiation and progression of all oral diseases is the formation of biofilms on the surfaces of the oral cavity (Marsh and Bradshaw, 1995). Current evidence supports the idea that pathogenic dental plaques do not form spontaneously. Instead, it has long been recognized that plaque formation is a temporally and spatially heterogeneous process, in which cariogenic plaques grow and change in response to environmental pressures (Theilade, 1990).

The key events in plaque formation are:

- Pellicle formation
- Colonization
- Maturation
ORAL BIOFILM FORMATION

ADHESION → COLONIZATION → COADHESION → MATURATION & DETACHMENT
Caries and Gingival Inflammation

Dental caries and periodontal disease, should be considered as consequences of ecologically driven imbalances of oral microbial biofilms (Marsh, 1994)
These ecologically driven changes in oral biofilms result in increases in the proportions of pathogenic microorganisms, which possess enzymatic and structural determinants that may render them more virulent than organisms associated with oral health.

The transition from health to disease is associated with compositional and metabolic changes in populations of bacteria that form the biofilms colonizing the hard and soft tissues of the mouth.

The development of dental caries is directly correlated with repeated cycles of plaque acidification, which encourage the emergence of aciduric bacteria at the expense of bacteria that are less acidogenic and less acid tolerant.
Acid production in dental plaque from fermentation of dietary carbohydrate lead to demineralization of the underlying enamel.
The oral cavity provides the habitat for a wide variety of microbial species. Fluctuations in the conditions in the oral cavity cause very rapid changes in availability of nutrient sources and pH values. Despite these apparently unfavourable milieu characteristics, approximately 500 microbial species colonize the oral cavity.

<table>
<thead>
<tr>
<th>Proportions of bacteria in developing supragingival plaque</th>
<th>Time of plaque development (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterium</td>
<td>2</td>
</tr>
<tr>
<td>S. sanguis</td>
<td>8</td>
</tr>
<tr>
<td>S. oralis</td>
<td>20</td>
</tr>
<tr>
<td>Mutans streptococci</td>
<td>3</td>
</tr>
<tr>
<td>S. salivarius</td>
<td>&lt;1</td>
</tr>
<tr>
<td>A. naeslundii</td>
<td>6</td>
</tr>
<tr>
<td>A. odontolyticus</td>
<td>2</td>
</tr>
<tr>
<td>Haemophilus spp.</td>
<td>11</td>
</tr>
<tr>
<td>Capnocytophaga spp.</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Fusobacterium spp.</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Black-pigmented anaerobes</td>
<td>0</td>
</tr>
</tbody>
</table>
Streptococci from all areas of the oral cavity are isolated and account for a large part of the oral resident flora.

In epidemiological studies streptococci were implied as the main pathogen of:
- enamel caries in children and young adults,
- root caries in the elderly population,
- and “Early Childhood Caries” in young children.

The oral streptococci are divided into four types:

<table>
<thead>
<tr>
<th>Mutans-group</th>
<th>Salivarius-group</th>
<th>Anginosus-group</th>
<th>Mitis-group</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. mutans,</td>
<td>S. salivarius,</td>
<td>S. constellatus</td>
<td>S. sanguis,</td>
</tr>
<tr>
<td>S. Sobrinus,</td>
<td>S. vestibularis</td>
<td>S. intermedius</td>
<td>S. gordonii,</td>
</tr>
<tr>
<td>S. rattus,</td>
<td></td>
<td>S. anginosus</td>
<td>S. parasanguis,</td>
</tr>
<tr>
<td>S. Ferus,</td>
<td></td>
<td></td>
<td>S. oralis,</td>
</tr>
<tr>
<td>S. downei</td>
<td></td>
<td></td>
<td>S. mitis,</td>
</tr>
</tbody>
</table>

The currently approved main groups of oral Streptococci with their species
Active carious lesions frequently do not contain high levels of S. mutans, but consistently harbor subpopulations of bacteria, mainly streptococci and lactobacilli, that are highly acidogenic and, more importantly, acid-tolerant (van Houte et al., 1994). While these findings do not exclude that mutans streptococci were involved in the initiation of disease, a lack of specificity in the etiology of the disease would diminish the value of targeting particular determinants of S. mutans.
The most relevant characteristic of acid-tolerant species in terms of caries development is that these organisms continue to grow and continue to produce acids at pH values well below that required for the dissolution of enamel (Bender et al., 1986; Bender and Marquis, 1987).
Biofilm management

- Dietary Aspects
- Inhibition of Colonisation
- Mechanical or Chemical Removal
Fermentable carbohydrates are implicated in the etiology of biofilm associated oral diseases, such as gingival inflammation and dental decay.

Therefore, artificial and natural sweeteners were introduced into preventive dental care to replace sucrose.
Oral Aspects of Steviosides - Prevention (Suppression of Biofilm)
Foods and beverages can be considered safe for teeth, if they are neither cariogenic nor erosive.

These "toothfriendly" properties are, for example, determined in standardized plaque-pH tests.
# Artificial sugar substitutes (examples)

(Many of these have little or no food energy. The sweetnesses is in comparison to those of sucrose.)

<table>
<thead>
<tr>
<th>Name</th>
<th>Sweetness (by weight)</th>
<th>Trade name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acesulfame potassium</td>
<td>200</td>
<td>Nutrinova</td>
</tr>
<tr>
<td>Aspartame</td>
<td>160–200</td>
<td>NutraSweet</td>
</tr>
<tr>
<td>Salt of aspartame-acesulfame</td>
<td>350</td>
<td>Twinsweet</td>
</tr>
<tr>
<td>Cyclamate</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Dulcin</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Glucin</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>Neotame</td>
<td>8,000</td>
<td>NutraSweet</td>
</tr>
<tr>
<td>Saccharin</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>Sucralose</td>
<td>600</td>
<td>Kaltame, Splenda</td>
</tr>
</tbody>
</table>
**Natural sugar substitutes** (examples)

(The sweetness is in comparison to those of sucrose.)

<table>
<thead>
<tr>
<th>Name</th>
<th>Sweetness by weight</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogenated starch hydrolysates</td>
<td>0.4–0.9</td>
<td></td>
</tr>
<tr>
<td>Lactitol</td>
<td>0.4</td>
<td>E966</td>
</tr>
<tr>
<td>Isomalt</td>
<td>0.45–0.65</td>
<td>E953</td>
</tr>
<tr>
<td>Mannitol</td>
<td>0.5</td>
<td>E421</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.6</td>
<td>E422</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>0.6</td>
<td>E420</td>
</tr>
<tr>
<td>Ervthritol</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Maltitol</td>
<td>0.9</td>
<td>E965</td>
</tr>
<tr>
<td>Xylitol</td>
<td>1.0</td>
<td>E967</td>
</tr>
<tr>
<td>Mabinlin</td>
<td>100</td>
<td>Protein</td>
</tr>
<tr>
<td>Stevia</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Pentadin</td>
<td>500</td>
<td>Protein</td>
</tr>
<tr>
<td>Curculin</td>
<td>550</td>
<td>Protein</td>
</tr>
<tr>
<td>Brazzein</td>
<td>800</td>
<td>Protein</td>
</tr>
<tr>
<td>Thaumatin</td>
<td>2,000</td>
<td>Protein; E957</td>
</tr>
</tbody>
</table>
The main sugar substitutes used are sorbitol and xylitol. Because it is not fermented by oral bacteria, xylitol is considered to be non-cariogenic, and while sorbitol in solution can be fermented slowly by mutans streptococci, chewing sorbitol-sweetened gum does not cause a fall in plaque pH.

Foods and beverages can be considered safe for teeth if they are neither cariogenic nor erosive.
Steviol glycosides are intense natural sweeteners that might have cariostatic potential. In current dental literature only a few reports were published dealing with possible dental health care effects of steviosides.

Yabu M, Takase M, Toda K, Tanimoto K, Yasutake A.
[Studies on Stevioside, natural sweetener. Effect on the growth of some oral microorganisms (author's transl)]
Hiroshima Daigaku Shigaku Zasshi. 1977; 9: 12-7. (Japanese, no abstract available.)

Ikeda T., Okada H. and Motoda R.
Effect of stevioside of certain metabolisms of Streptococcus mutans.

Berry CW, Henry CA.
Steviol glycosides are intense natural sweeteners that may have cariostatic potential. In current dental literature only a few reports were published dealing with possible dental health care effects of steviosides.

Ikeda T.

Pinheiro CE, de Oliveira SS, da Silva SM, Poletto MI, Pinheiro CF.
Effect of guaraná and Stévia Rebaudiana Bertoni (leaves) extracts, and stevioside, on the fermentation and synthesis of extracellular insoluble polysaccharides of dental plaque

Kleber CJ.
Rat dental caries investigation of stevioside natural sweetener. Benton, Sun Up Foods, **1990**. final report, no abstract available
Steviol glycosides are intense natural sweeteners that may have a cariostatic potential. In current dental literature only a few reports were published dealing with possible dental health care effects of steviosides.


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Summary:
The cariostatic effects of Steviol glycosides were only investigated in 2 international publications (Berry et al. 1981 and Das et al. 1992):

- S. mutans: reduction in growth and decrease of acid production (in vitro)
- Reduction of caries in rats colonized with S. sobrinus
Aim of the Investigations:

Evaluation of the effects of steviol glycosides on the growth rate and acid production of caries / oral biofilm related species compared to fermentable carbohydrates.
Preliminary Results of In Vitro Tests: Material and Methods

Protocol of the In Vitro Investigation:

- Primary culture of test species
- Inoculation of 50µl test species suspension to test medium containing sugar or sugar substitutes
- Measurement of growth and acid production during 52 hour period
Preliminary results of in-vitro tests: Material and Methods

Biofilm associated oral test species:

- S. mutans Clarke 1924 (DSM 20532)
- S. Sobrinus (DSM 20742)
- S. sanguis (DSM 20068)
- S. salivarius (DSM 20067)

Purity was controlled by: BD Phoenix™
Medium Composition and Preparation

“Brain-Heart-Infusion” (Heipha Dr. Müller GmbH, REF: 3110r) was used as basic media for the bacterial culture.

The composition of this media per litre is:

- Brain Infusion (12.5g)
- Phosphate (2.5g)
- Heart Infusion (5g)
- Glucose (2g)
- Peptone (10g)
- NaCl (5g)
- pH 7.4 +/- 0.2

For the measurements either sucrose or glucose in additional with either stevioside or rebaudioside A were added to the media.
Preliminary Results of In Vitro Tests: Material and Methods

Medium Composition and Preparation

<table>
<thead>
<tr>
<th>Sugar/Sugar substitute</th>
<th>Stock [%]</th>
<th>Stock</th>
<th>Added amount of Stock solution (filled up with “Brain-Heart-Infusion” to 50ml) [μl/50ml]</th>
<th>Final concentration [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>50</td>
<td>5g/10ml</td>
<td>500</td>
<td>0.5</td>
</tr>
<tr>
<td>Sucrose + Stevioside</td>
<td>50/50</td>
<td>5g/10ml</td>
<td>500</td>
<td>0.5 &amp; 0.5</td>
</tr>
<tr>
<td>Sucrose + Rebaudioside</td>
<td>50/0.4</td>
<td>5g/10ml &amp; 200mg/50ml</td>
<td>500 &amp; 5000</td>
<td>0.5 &amp; 0.04</td>
</tr>
<tr>
<td>Stevioside</td>
<td>50</td>
<td>5g/10ml</td>
<td>500</td>
<td>0.5</td>
</tr>
<tr>
<td>Rebaudioside</td>
<td>0.4</td>
<td>200mg/50ml</td>
<td>5000</td>
<td>0.04</td>
</tr>
<tr>
<td>Glucose</td>
<td>50</td>
<td>5g/10ml</td>
<td>500</td>
<td>0.5</td>
</tr>
<tr>
<td>Glucose + Stevioside</td>
<td>50/50</td>
<td>5g/10ml &amp; 5g/10ml</td>
<td>500 &amp; 500</td>
<td>0.5/0.5</td>
</tr>
<tr>
<td>Glucose + Rebaudioside</td>
<td>50/0.4</td>
<td>5g/10ml &amp; 200mg/50ml</td>
<td>500 &amp; 5000</td>
<td>0.5/0.04</td>
</tr>
</tbody>
</table>

Shown is the preparation of the medium (“Brain-Heart-Infusion”) from stock solutions [%] to the final concentrations [%], to get different constituted media containing additional sucrose, glucose, stevioside or rebaudioside A (sugar substitutes) or a combination of two of these constitutes.
Preliminary Results of In Vitro Tests: Material and Methods

Measurement of Optical Density:
Optical density was measured at 600nm using a photometer (Biochrom Ltd, WPA CO Biowave 8000 Cell Density Meter).

Measurement of pH:
A standard pH-measurement unit was used (WTW GmbH, Profilab pH 597)
Experimental Protocol

1. Fresh bacterial culture adjusted to OD 0.8 - 1.0 with added „Brain-Heart-Infusion“
2. 50µl of the OD adjusted solution was added to 5ml medium
3. Incubation of sample at 37°C in a shaken water bath during a 52 hour period
4. Measurement of optical density and pH
   - OD: hourly [0-24], 28, 32, 40, 44, 48 and 52 h
   - pH : 2 h [0-24], 28, 32, 40, 44, 48 and 52 h
5. Replication of each parameter selection: n=5
Preliminary Results of In Vitro Tests: Results (Glucose)

S. sanguis

- Negative control
- Glucose 0.5%
- Glucose 0.5% + Stevioside 0.5%
- Glucose 0.5% + Rebaudioside A 0.04%
- Stevioside 0.5%
- Rebaudioside A 0.04%

n=5

median of OD
Preliminary Results of In Vitro Tests: Results (Glucose)

Minor pH decline in the stevioside/rebaudioside A medium
Preliminary Results of In Vitro Tests: Results (Glucose)

- Negative control
- Glucose 0.5%
- Glucose 0.5% + Stevioside 0.5%
- Glucose 0.5% + Rebaudioside A 0.04%
- Stevioside 0.5%
- Rebaudioside A 0.04%

n=5
median of OD

Graph showing growth over time for different conditions.
Preliminary Results of In Vitro Tests: Results (Glucose)

Minor pH decline in the stevioside/rebaudioside A medium
**Preliminary Results of In Vitro Tests: Results (Glucose)**

- **Negative control**
- **Glucose 0.5%**
- **Glucose 0.5% + Stevioside 0.5%**
- **Glucose 0.5% + Rebaudioside A 0.04%**
- **Stevioside 0.5%**
- **Rebaudioside A 0.04%**

**S. sobrinus**

- Median of OD
- **n=5**

Graph showing the growth of *S. sobrinus* over time with different treatments.
**Preliminary Results of In Vitro Tests: Results (Glucose)**

**S. sobrinus**

- Negative control
- Glucose 0.5%
- Glucose 0.5% + Stevioside 0.5%
- Glucose 0.5% + Rebaudioside A 0.04%
- Stevioside 0.5%
- Rebaudioside A 0.04%

n=5
median of pH

Minor pH decline in the stevioside/rebaudioside A medium
Preliminary Results of In Vitro Tests: Results (Glucose)

S. mutans

- Negative control
- Glucose 0.5%
- Glucose 0.5% + Stevioside 0.5%
- Glucose 0.5% + Rebaudioside A 0.04%
- Stevioside 0.5%
- Rebaudioside A 0.04%

n=5
median of OD
Preliminary Results of In Vitro Tests: Results (Glucose)

Minor pH decline in the stevioside/rebaudioside A medium
Preliminary Results of In Vitro Tests: Results

Minor pH decline in the stevioside/ rebaudioside A medium

n=5
median of OD and pH
Preliminary Results of In Vitro Tests: Results (Sucrose)

Minor pH decline in the stevioside/rebaudioside A medium

n=5
median of OD and pH
Preliminary Results of In Vitro Tests: Results (Sucrose)

Aggregation of S. sobrinus

Minor pH decline in the stevioside/ rebaudioside A medium

n=5
median of OD and pH
Preliminary Results of In Vitro Tests: Results (Sucrose)

Aggregation of S. mutans

Minor pH decline in the stevioside/rebaudioside A medium

n=5
median of OD and pH
Conclusion:

The samples containing stevioside or rebaudioside A showed a reduced pH-decrease even in the presence of carbohydrates.

In S. sobrinus and S. mutans-cultures after a certain time aggregates were observed influencing the OD-measurements.
• Despite a certain caries decline in European countries carbohydrates represent one of the most important factors for oral diseases.

• For example, the cost of dental treatment in Germany amounts to >11 billions € per year (2009).

• Dental health care (including caries preventive diet) is the strongest strategy in dentistry supported by all health care organizations.

• In modern lifestyle perfect teeth are growing in significance.
Estimated Advantages of Steviosides in Dentistry

• Sugar substitutes are well accepted, and evidence based adjuvants in dental health care.

• Natural agents are highly accepted by consumers. Due to current scientific results Stevia as a sugar substitute has cariostatic properties. This product offers unique future possibilities in the marketing field.
Example for new marketing activities:

**Stevia Is Toothfriendly-Approved**

Toothfriendly International, a non-profit association governed by dental professionals, has given its seal of approval to Wild's Sunwin Stevia based on a plaque-pH telemetry study conducted at the Dental Institute of the University of Zürich. The study demonstrated that Wild's Stevia sweetener poses no risk for teeth, as it does not suppress the plaque-pH below the critical value of 5.7. When the acid threshold falls below this threshold, teeth can be damaged.

The Toothfriendly approval means that food manufacturers can use Wild's Sunwin Stevia as a guaranteed toothfriendly ingredient within the formula of their final product.
Estimated Advantages of Steviosides in Dentistry

• The biological effects of steviol glycosides to oral biofilms are mostly unknown. Only a few scientific investigations are related to this subject.

• Steviol glycosides may even counteract the pH-drop in biofilms and therefore, Stevia may have preventive properties. This preventive potential needs to be scientifically verified.

• The caries protective aspects of Stevia would enhance acceptance as an oral health-care product and open new marketing strategies.
Acknowledgements

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Thank you!